

Amendments to the Specification:

Please replace the paragraph beginning at page 8, line 7, with the following:

--Figure 1 shows the effect of Ara-C (350 mg/kg) on White Blood Cell Count (WBC) in mice in the presence (triangular data points, solid line, designated Ara-C + CTCE0021 (SEQ ID NO:23) in the legend) and absence (circular data points, dashed line, designated Ara-C in the legend) of a peptide of the invention (designated CTCE0021 (SEQ ID NO:23) and described in Examples 1 and 3).--

Please replace the paragraph beginning at page 8, line 13, with the following:

--Figure 2A shows a concentration-dependant inhibition of ^{125}I -SDF-1 binding to CXCR4 by SDF-1 (SEQ ID NO:1), obtained as described for the data shown in Figure 2A, indicating the affinity of SDF-1 (SEQ ID NO:1) for the CXCR4 receptor.--

Please replace the paragraph beginning at page 8, line 17, with the following:

--Figure 2B shows the CXCR4 receptor binding of SDF-1 (SEQ ID NO:1) and the SDF-1 peptide agonist analogs. SDF-1 (SEQ ID NO:1) and the indicated analogs (competing ligands, described in Examples) were added at the concentrations illustrated in the presence of 4nM ^{125}I -SDF-1. CEM cells were assessed for ^{125}I -SDF-1 binding following 2 hr of incubation. The results are expressed as percentages of the maximal specific binding that was determined without competing ligand, and are the mean of three independent experiments.--

Please replace the paragraph beginning at page 8, line 25, with the following:

--Figure 3 shows the induction of $[Ca^{2+}]_i$ mobilization by SDF-1 (SEQ ID NO:1) and SDF-1 receptor analogs (described in Examples). Fura-2,AM loaded THP-1 cells (1×10^6 /ml) were stimulated with SDF-1 (SEQ ID NO:1), CTCE0021 (SEQ ID NO:23) or CTCE0022 (SEQ ID NO:22) at the concentrations indicated. The values represent the mean +/- one S.D. of n=3 experiments.--

Please replace the paragraph beginning at page 8, line 31, with the following:

--Figure 4 shows the induction of $[Ca^{2+}]_i$ mobilization by SDF-1 (SEQ ID NO:1) and SDF-1 analogs. Fura-2,AM loaded THP-1 cells (1×10^6 /ml) were stimulated with native SDF-1 (SEQ ID NO:1) and the SDF-1 peptide analogs at the concentration of native SDF-1 concentration that gave the maximum $[Ca^{2+}]_i$ stimulation ($1 \mu M$). The values represent the mean +/- one S.D. of n=3 experiments. The designated compounds are as follows: SDF-1 (SEQ ID NO:1), SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021) (SEQ ID NO:23), SDF-1(1-14)-(G)₄-SDF-1(55-67)-E24/K28-cyclic amide (CTCE0022) (SEQ ID NO:22), SDF-1(1-9)₂-C9/C9-cysteine dimer (CTCE9901) (SEQ ID NO:7), SDF-1(1-17) (CTCE9902) (SEQ ID NO:4), SDF-1(1-8)₂-lysine bridge dimer (CTCE9904) (SEQ ID NOS:31 and 32) and SDF-1(1-14)-(G)₄-SDF-1(55-67) amide (CTCE0017) (SEQ ID NO:15).--

Please replace the paragraph beginning at page 9, line 18, with the following:

--Figure 6 shows cyclic proliferative activity of primitive normal CFC in the adherent layer of standard LTC, when treated with SDF-1 (SEQ ID NO:1), SDF-

1(1-14)-(G)₄-SDF-1(55-67)-K20/D24-cyclic amide (Compound #1) (SEQ ID NO:24), SDF-1(1-9)₂ (Compound #3) (SEQ ID NOS:8 and 9), as measured by the susceptibility of the cells to an agent preferentially cytotoxic to dividing cells.--

Please replace the paragraph beginning at page 9, line 24, with the following:

--Figure 7 shows the effect of SDF-1 (SEQ ID NO:1) and SDF-1 analogs (defined in Examples) on the cycling of human progenitors from fetal liver transplanted NOD/SCID mice. The cycling status of mature and primitive colony forming cells (CFU-GM; colony forming unit-granulocyte-monocyte precursor, BFU-E; burst forming unit-erythroid precursor) in the suspension of CD34⁺ cells isolated from the marrow of transplanted NOD/SCID mice was determined by assessing the proportion of these progenitors that were inactivated (killed) by short term (20 min) or overnight (LTC-IC; long-term culture initiating cell) exposure of the cells to 20µg/ml of high specific activity ³H-thymidine. Values represent the mean +/- the S.D. of data from up to four experiments with up to four mice per point in each.--

Please replace the paragraph beginning at page 10, line 8, with the following:

--Figure 9 shows the effect of SDF-1 (SEQ ID NO:1) and SDF-1 Agonists (defined in Examples) on the engraftment of human cells in human fetal liver transplanted NOD/SCID mice. A comparison of the number of phenotypically defined hematopoietic cells detected in the long bones (tibias and femurs) of mice four weeks after being transplanted with 10⁷ light-density human fetal liver blood cells and then administered SDF-1 (SEQ ID NO:1), CTCE0021 (SEQ ID NO:23) or CTCE 0013 (SEQ ID NO:13) (0.5 mg/kg) three times per week for two

weeks before sacrifice. Values represent the mean +/- one S.D. of results obtained from three to seven individual mice in three experiments.--

Please replace the paragraph beginning at page 10, line 18, with the following:

--Figure 10 shows the effect of CTCE0021 (SEQ ID NO:23) (1mg/kg, defined in the Examples) on the recovery of leukocytes following myeloablative chemotherapy with Ara-C (300mg/kg). Mice were treated with Ara-C alone (Ara-C) or in combination with CTCE0021 (SEQ ID NO:23). The results represent the mean +/- one S.D. of 6 animals/group.--

Please replace the paragraph beginning at page 10, line 24, with the following:

--Figure 11 shows the effect of CTCE0021 (SEQ ID NO:23) (defined in Examples) and Neupogen[®] (G-CSF) on the growth of white blood cells in Ara-C treated mice. C3Hhen mice (female) were treated with 500mg/kg Ara-C for two cycles - on days 0 and 10. During the second cycle of Ara-C dosing, Ara-C treated mice were injected with 10mg/kg CTCE0021 (SEQ ID NO:23), 10mg/kg Neupogen[®], alone or together (on days -1, 0, and 1 to 3). Control represents animals treated with Ara-C alone. Blood was collected from the tail vein into heparin-containing tubes at the onset of the experiment, and one day before and 1, 7 and 12 days following the second Ara-C dose. A total white blood cell count was obtained. The results represent the mean +/- one S.D. of 6 animals/group.--

Please replace the paragraph beginning at page 11, line 4, with the following:

--Figure 12 shows the effect of CTCE0021 (SEQ ID NO:23) and Neupogen[®] on the relative growth of white blood cells in Ara-C treated mice.

C3Hhen mice (female) were treated with 500mg/kg Ara-C for two cycles - on days 0 and 10. During the second cycle of Ara-C dosing, Ara-C treated mice were injected with 10mg/kg CTCE0021 (SEQ ID NO:23) (defined in Examples), 10mg/kg Neupogen[®], alone or together (on days -1, 0, and 1 to 3). Control represents animals treated with Ara-C alone. Blood was collected from the tail vein into heparin-containing tubes at the onset of the experiment, and one day before 7 and 12 days following the second Ara-C dose. A total white blood cell count was obtained. The increase in leukocytes (white blood cells) was determined relative to the number of cells counted the day before the second cycle Ara-C dose was administered. The results represent the mean +/- one S.D. of 6 animals/group.--

Please replace the paragraph beginning at page 11, line 18, with the following:

--Figure 13 shows the sequences of human SDF-1alpha (SEQ ID NO:1), SDF-1 Precursor (PBSF) (SEQ ID NO:2) and SDF-1beta (SEQ ID NO:3).--

Please replace the paragraph beginning at page 14, line 24, with the following:

--In some embodiments, the present invention is concerned with polypeptides having the amino acid sequences shown in ~~SEQ ID. NO.'s 1, 2 or 3~~ SEQ ID NOS:1, 2 or 3 (Figure 13). In some embodiments, a pegylation moiety may be provided at any position on the sequence. The polypeptides of the present invention may include polypeptides in which part of the amino acid sequence is omitted, or polypeptides that consist of sequences containing additional or replaced amino acids, or spliced forms of the sequences, yet induce activation of the CXCR4. In some embodiments, polypeptides of the invention may be at least 70%, 80% 90% or 95% identical to the polypeptides of ~~Seq. ID.~~

~~No.'s 1, 2 or 3~~ SEQ ID NOS:1, 2 or 3, when optimally aligned, over a region of at least 10, 15, 20, 30, 40, 50, 60 or 80 or more, contiguous amino acids. In alternative embodiments, SDF-1 polypeptides of the invention may consist of amino acid sequences that are less than 70% identical to portions of ~~SEQ ID No.'s 1, 2 or 3~~ SEQ ID NOS:1, 2 or 3, where a polypeptide demonstrates CXCR4 agonist activity, such as activity that is comparable (such as within 0.01-, 0.1-, 1.0-, 10-, or 100-fold) to that obtained with the SDF-1 polypeptides of ~~Seq. ID. No.'s 1, 2 or 3~~ SEQ ID NOS:1, 2 or 3.--

Please replace the paragraph beginning at page 15, line 9, with the following:

--In one aspect of the invention, a putative SDF-1 polypeptide having some similarity to ~~SEQ ID No.'s 1, 2 or 3~~ SEQ ID NOS:1, 2 or 3 may be assessed for CXCR4 agonist activity. Putative SDF-1 polypeptides of the invention may for example be assayed for CXCR4 receptor binding as determined by receptor binding assays, such as radiolabeled ligand receptor competition assays. Activation of CXCR4 by an SDF-1 polypeptide may be determined through assaying activation of the receptor through standard biochemical techniques including intracellular calcium mobilization, cellular chemotaxis, chemiluminescence, degranulation assays, measurement of NADPH-dependent formation of reactive oxygen species, activation of secondary messenger enzymes such as G proteins, phospholipase C (PLC), protein kinase C (PKC), or of Src and Src family kinases (i.e., Fyn). In some embodiments, CXCR4 agonist activity, CXCR4 receptor binding or CXCR4 receptor activation of a putative CXCR4 agonist of the invention may be at least 0.01-, 0.1-, 1.0-, 10-, or 100-fold of the corresponding parameter of a polypeptides of ~~Seq. ID. No.'s 1, 2 or 3~~ SEQ ID NOS:1, 2 or 3.--

Please replace the paragraph beginning at page 16, line 24, with the following:

--In some embodiments, CXCR4 agonist peptides may for example also be selected from the group consisting of peptides having the following sequences:

a) SDF-1-derived cyclic amide (E24/K28) agonists (such as CTCE0021 (SEQ ID NO:23)) having the formula:

[RNH-Lys]XaaVSXbbSYRCPCRFF[linker]LKWIQEYLEKALN-NH₂ (SEQ ID NOS:35, 204 and 205); and

b) SDF-1-derived cyclic acid (K20/E24) agonists (such as CTCE0022 (SEQ ID NO:22)) having the formula:

[RCONH-Lys]XaaVSXbbSYRCPCRFF[linker]LKWIQEYLEKALN-NH₂ (SEQ ID NOS:36, 206 and 207);

wherein, R is a substituent that may for example be a hydrogen, alkyl, aryl or polyethyleneglycol (PEG) moiety; Xaa is an amino acid that may for example be either an L-Proline or a D-Proline moiety; Xbb is an amino acid that may for example be either a L-Leucine or a D-Leucine moiety; and [linker] is a moiety providing a covalent attachment between the N and C terminal portions of the peptides, such as a linking moiety having 4 glycines (SEQ ID NO:211) or NH₂-(CH₂)_n-COOH (n=0-20).--

Please replace the paragraph beginning at page 44, line 1, with the following:

--In accordance with various aspects of the invention, a wide variety of peptide sequences may be prepared, for which the following nomenclature may be used. The portions of the peptide corresponding to a chemokine sequence, such as an SDF-1 sequence may be identified by specifying the corresponding portion of the chemokine, wherein for example a reference to an SDF-1 sequence refers to a sequence having substantial identity to a portion of the

sequence of ~~SEQ ID No: 1~~ SEQ ID NO:1. For example, the nomenclature SDF-1(1-14) connotes the first fourteen amino acids of the N-terminal sequence of SDF-1 of ~~SEQ ID No: 1~~ SEQ ID NO:1. In some embodiments, N-terminal and C-terminal portions of an SDF-1 sequence may be linked by various amino acids, or other linking moieties, denoted by a formula (L)_n, wherein "L" is a linking moiety which may for example be an amino acid and n is zero or an integer. The carboxy terminal of the peptide may be modified to be an amide rather than a carboxylic acid. In some embodiments, polypeptides of the invention may be of the following formula:

SDF-1(1-X)-(L)_n-SDF-1(Y-Z)

wherein:

X is an integer from 5 to 20;

L is a linking moiety having at least one carbon atom, such as a substituted or unsubstituted alkyl moiety, or an amino acid;

n is an integer from 1 to 50

Y is an integer from 50 to 60

Z is an integer from 60 to 67.--

Please replace the paragraph beginning at page 46, line 8, with the following:

--In alternative embodiments of the peptides of the invention, underlined spacer monomers (such as the illustrated glycine G's) may ~~for~~ be used in variable numbers, such as 2, 3 or 4 glycines (SEQ ID NO:214).--

Please replace the paragraph beginning at page 46, line 12, with the following:

--In alternative embodiments, internal cyclization of peptides of the invention may be in alternative positions, or between substituted amino acids.

The nature of the cyclic linkage may also be varied. For example, the linkage may be shortened by replacing the relevant glutamate (E) with an aspartate (D) residue, and/or replacing the lysine (K) with an ornithine (O) residue. Cyclization is for example possible between Aspartic acid 24 (D24) and Lysine 20 or 28 (K20 or K28), as illustrated in some of the exemplified polypeptides.

SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/D24-cyclic acid

H₂NKPVSLSYRCPCRFFGGGGLKWIQDYLEKALNCOOH (SEQ ID NO:24)

H₂NKPVSLSYRCPCRFFGGGGLKWIQDYLEKALNCOOH (SEQ ID NO:24)

SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/D24-cyclic amide

H₂NKPVSLSYRCPCRFFGGGGLKWIQDYLEKALNCONH₂ (SEQ ID NO:25)

H₂NKPVSLSYRCPCRFFGGGGLKWIQDYLEKALNCONH₂ (SEQ ID NO:25)--

Please replace the paragraph beginning at page 47, line 19, with the following:

--As shown above, exemplary embodiments of polypeptides of the invention have been synthesized, having N-terminal SDF-1 residues (1-14) or (1-17), linked to C-terminal SDF-1 residues (55-67) by a three or four-glycine linker (SEQ ID NO:212). In some embodiments, peptides are cyclized between glutamic acid (at 24 position) and lysine (at 20 or 28 position). Lactamization may be affected by removing the allylic group from both side chains of lysine and glutamic acid using the palladium technique and then effecting internal amide bond formation between the corresponding lysine and glutamic acid. Selected members of this family of polypeptides showed high affinity in a CXCR4 receptor binding assay (CEM cells) and in activating [Ca²⁺] mobilization (THP-1 cells). Further embodiments of polypeptides are listed below:

SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/D24-(E24 ? D)-cyclic acid or amide
(SEQ ID NOS:24 and 25)

SDF-1(1-14)-(G)₄-SDF-1(55-67)-K28/D24-(E24 ? D)-cyclic acid or amide
(SEQ ID NOS:37 and 38)

Cyclization may also take place between ornithine (O) and glutamic acid (E):

SDF-1(1-14)-(G)₄-SDF-1(55-67)-O20/E24-(K20 ? O)-cyclic acid or amide
(SEQ ID NOS:39 and 40)

SDF-1(1-14)-(G)₄-SDF-1(55-67)-O28/E24-(K28 ? O)-cyclic acid or amide
(SEQ ID NOS:41 and 42)

Cyclization may also take place between ornithine (O) and aspartic acid (D):

SDF-1(1-14)-(G)₄-SDF-1(55-67)-O20/D24-(K20 ? O & E24 ? D)-cyclic acid or amide
(SEQ ID NOS:43 and 44)

SDF-1(1-14)-(G)₄-SDF-1(55-67)-O28/D24-(K28 ? O & E24 ? D)-cyclic acid or
amide (SEQ ID NOS:45 and 46)--

Please replace the paragraph beginning at page 49, line 11, with the following:

--Similarly, polypeptides may be prepared using sequences from chemokines
other than SDF-1. Such as residues 36-50, 10-50 or 55-70 of MIP-1 α :

SDF-1(1-14)-(G)₄-MIP-1 α (36-50)-acid or amide

H₂N-KPVLSYRCPCRFFGGGGSKPGVIFLTRSRQV-CONH₂ (~~SEQ ID NO:28~~)

(SEQ ID NOS:28 and 47)

SDF-1(1-14)-(G)₄-MIP-1 α (11-50)- acid or amide

H₂N-

KPVLSYRCPCRFFGGGGCCFSYTSRQIPQNFIADYFETSSQCSKPGVIFLTR

SRQV-CONH₂ (~~SEQ ID NO:29~~) (SEQ ID NOS:29 and 48)

SDF-1(1-14)-(G)₄-MIP-1 α (56-70)-acid or amide

H₂N-KPVLSYRCPCRFFGGGGEEWVQKYVDDLELSA-CONH₂ (~~SEQ ID~~

~~NO:30~~) (SEQ ID NOS:30 and 49)--

Please replace the paragraph (and Table 2) beginning at page 52, line 1, with the following:

--Table 2 further demonstrates that SDF-1(1-14)-(G)₄-SDF-1(55-67)-amide (SEQ ID NO:15) and SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (SEQ ID NO:23) are both able to inhibit cell cycling in human positive erythroid and primitive granulopoietic cells, but not in mature granulopoietic cells, in the assay as described above in this Example.

Table 2

	% CELL KILLED		
	No drug (control)	Compound A	Compound B
Primitive Erythroide	47 +/- 4	5 +/- 3	-7 +/- 6
Primitive Granulocyte	42 +/- 3	1 +/- 6	-11 +/- 7
Mature Granulocyte	48 +/- 3	39 +/- 5	44 +/- 6

Where: Compound A is SDF-1(1-14)-(G)₄-SDF-1(55-67)-amide (SEQ ID NO:15);

Compounds Compound B is SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (SEQ ID NO:23).--

Please replace the paragraph beginning at page 52, line 20, with the following:

--As shown in the graph of Figure 1, in mice given a single dose of Arabinose Cytosine (Ara-C) at 350 mg/kg at day zero intravenously, white blood cell count (WBC) decreases (due to the cytotoxic action of Ara-C). In contrast, in mice given the peptide SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (SEQ ID NO:23) (designated CTC in the graph legend) in combination with Ara-C, the extent of white blood cell count decrease is significantly ameliorated. In the graph, circular data points correspond to the white blood cell count in animals that received Ara-C but did not receive the peptide, and triangular data points are for animals that received Ara-C and SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (SEQ ID NO:23). The data clearly demonstrated the protective action of the peptide of the invention against the cytotoxic action of Ara-C.--

Please replace the paragraph beginning at page 53, line 7, with the following:

--The efficacy of SDF-1 and SDF-1 peptide analogs as CXCR4 agonists was demonstrated through CXCR4 receptor binding assays. A competitive dose response for binding to the SDF-1 receptor by native SDF-1 and the CXCR4 agonists against ^{125}I -SDF-1 is shown in Figures 2A and 2 B respectively. A concentration-dependent inhibition of ^{125}I -SDF-1 is illustrated in Figure 2A, indicating the affinity of SDF-1 for the receptor. A Scatchard plot is illustrated, and the K_D was determined to be 26nM. SDF-1 and the indicated analogs (competing ligands) were added at the concentrations illustrated in the presence of 4nM ^{125}I -SDF-1. CEM cells were assessed for ^{125}I -SDF-1 binding following 2 hr of incubation. The results are expressed as percentages of the maximal specific binding that was determined without competing ligand, and are the mean of three independent experiments. The inhibition of ^{125}I -SDF-1 by SDF-1 and the SDF-1 analogs is indicative of CXCR4 receptor binding. The compounds illustrated in the figure are as follows: SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021) (SEQ ID NO:23), SDF-1(1-14)-(G)₄-SDF-1(55-67)-E24/K28-cyclic amide (CTCE0022) (SEQ ID NO:22), SDF-1 (1-9)₂-C9/C9-cysteine dimer (CTCE9901) (SEQ ID NO:7), SDF-1(1-17) (CTCE9902) (SEQ ID NO:4), SDF-1 (1-8)₂-lysine bridge dimer (CTCE9904) (SEQ ID NOS:31 and 32) and SDF-1(1-14)-(G)₄-SDF-1(55-67) amide (CTCE0017) (SEQ ID NO:15).--

Please replace the paragraph (and Table 3) beginning at page 53, line 28, with the following:

--This example illustrates the efficacy of SDF-1 and SDF-1 peptide analogs in mediating intracellular calcium mobilization ($[\text{Ca}^{2+}]_i$). To illustrate that the binding of SDF-1 and SDF-1 peptide analogs results in the agonistic induction of the CXCR4 receptor, $[\text{Ca}^{2+}]_i$ mobilization assays were conducted, the results of which are shown in Figure 3. To obtain the data shown in Figure 3,

fura-2,AM loaded THP-1 cells (1×10^6 /ml) were stimulated with SDF-1 (SEQ ID NO:1), SDF-1(1-14)-(G)₄-SDF-1(55-67)K20/E24-cyclic amide (SEQ ID NO:23) or SDF-1(1-14)-(G)₄-SDF-1(55-67)-E24/K28-cyclic amide (SEQ ID NO:22) at the concentrations indicated (the values represent the mean +/- one S.D. of n=3 experiments). As shown by the data in Figure 3, incubation of THP-1 cells with SDF-1 (SEQ ID NO:1), SDF-1(1-14)-(G)₄-SDF-1(55-67)K20/E24-cyclic amide (SEQ ID NO:23) or SDF-1(1-14)-(G)₄-SDF-1(55-67)-E24/K28-cyclic amide (SEQ ID NO:22) resulted in the receptor-mediated induction of $[Ca^{2+}]_i$ mobilization. The EC₅₀ values (the concentration of ligand necessary to effectively induce 50% of the full $[Ca^{2+}]_i$ mobilization potential) for SDF-1(1-14)-(G)₄-SDF-1(55-67) acid (SEQ ID NO:13), SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (SEQ ID NO:23) or SDF-1(1-14)-(G)₄-SDF-1(55-67)-E24/K28-cyclic amide (SEQ ID NO:22) and native SDF-1 (SEQ ID NO:1) is shown in Table 3:

Table 3

Compound	EC ₅₀ (nM)
SDF-1 (<u>SEQ ID NO:1</u>)	26.56
SDF-1(1-14)-(G) ₄ -SDF-1(55-67)-E24/K28-cyclic amide (<u>SEQ ID NO:22</u>)	106.25
SDF-1(1-14)-(G) ₄ -SDF-1(55-67)-K20/E24-cyclic amide (<u>SEQ ID NO:23</u>)	147.94
SDF-1(1-14)-(G) ₄ -SDF-1(55-67) acid (<u>SEQ ID NO:13</u>)	188.30

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Please replace the paragraph beginning at page 54, line 16, with the following:

--The comparative ability of SDF-1 (SEQ ID NO:1), SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021) (SEQ ID NO:23), SDF-1(1-14)-(G)₄-SDF-1(55-67)-E24/K28-cyclic amide (CTCE0022) (SEQ ID NO:22), SDF-1(1-9)₂-C9/C9-cysteine dimer (CTCE9901) (SEQ ID NO:7), SDF-1(1-17) (CTCE9902) (SEQ ID NO:4), SDF-1 (1-8)₂-lysine bridge dimer (CTCE9904)

(SEQ ID NOS:31 and 32) and SDF-1(1-14)-(G)₄-SDF-1(55-67) amide (CTCE0017) (SEQ ID NO:15) to induce $[Ca^{2+}]_i$ mobilization at the ligand concentration that the native SDF-1 gave maximal $[Ca^{2+}]_i$ mobilization (1 μ M, refer to Figure 3) is illustrated in Figure 4. Fura-2,AM loaded THP-1 cells (1x10⁶/ml) were stimulated with native SDF-1 and the SDF-1 peptide agonist analogs at the concentration of native SDF-1 that gave the maximum $[Ca^{2+}]_i$ stimulation (1 μ M) (the values represent the mean +/- one S.D. of n=3 experiments).--

Please replace the paragraph (and Table 4) beginning at page 55, line 16, with the following:

--The results depicted in Table 4 illustrate the ability of SDF-1 (SEQ ID NO:1), and SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021) (SEQ ID NO:23) and SDF-1(1-14)-(G)₄-SDF-1(55-67) acid (CTCE0013) (SEQ ID NO:13) to repress the proliferation of clonogenic erythroid and granulopoietic progenitors (which differentiate into erythrocytes, platelets, monocytes/macrophages and neutrophils) in an *in vitro* LTC-IC (long-term culture-initiating cells) assay.

Table 4. Effect of SDF-1 and SDF-1 agonists on the cycling of primitive progenitors in the adherent layer of human LTC.

		<u>% Kill after ³H-</u>	
<u>Thymidine</u>			
<u>Treatment</u>	<u>Dose</u>	<u>Primitive BFU-E</u>	<u>Primitive CFU-GM</u>
None		48 +/- 4	44 +/- 3
CTCE0013 (SEQ ID NO:13)	1 µg/ml	24 +/- 6	22 +/- 7
	10 µg/ml	0 +/- 2	0 +/- 0
SDF-1 (SEQ ID NO:1)	1 µg/ml	4 +/- 3	5 +/- 4
CTCE0021 (SEQ ID NO:23)	1 µg/ml	2 +/- 4	0 +/- 3--

Please replace the paragraph beginning at page 56, line 12, with the following:

--Figure 5 illustrates that feeding cultures SDF-1 in conjunction with media changes results in significantly reduced cell mortality of hematopoietic cells when the cells are challenged with an agent that is preferentially cytotoxic to dividing cells, in which circles represent BFU-E cells (burst forming unit--erythroid precursors), and squares represent CFU-GM cells (colony forming unit--granulocyte-monocyte common precursor). Figure 6 shows that a similar concentration dependent effect may be obtained with SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/D24-cyclic amide (Compound #1) (SEQ ID NO:25) and SDF-1(1-9)₂ (Compound #3) (SEQ ID NOS:8 and 9). Together, Figures 5 and 6 illustrate that the SDF-1 (SEQ ID NO:1) polypeptide and SDF-1 peptide analogs repress the cyclic activation of the BFU-E and CFU-GM progenitor stem cells in the adherent layer of LTC.--

Please replace the paragraph beginning at page 56, line 29, with the following:

--In Figure 7, the cycling status of mature and primitive colony forming cells (CFU-GM; colony forming unit-granulocyte-monocyte precursor, BFU-E; burst forming unit-erythroid precursor; LTC-IC, long-term culture initiating cell) in the suspension of CD34⁺ cells isolated from the marrow of transplanted NOD/SCID mice was determined by assessing the proportion of these progenitors that were inactivated (killed) by short term (20 min) or overnight (16 hour) exposure of the cells to 20µg/ml of high specific activity ³H-thymidine (values represent the mean +/- the S.D. of data from up to four experiments with up to four mice per point in each). Significant in the results described in Figure 4 is the observation that the analogs SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021) (SEQ ID NO:23) and SDF-1(1-14)-(G)₄-SDF-1(55-67) acid (CTCE013) (CTCE0013) (SEQ ID NO:13) are as effective as native SDF-1 (SEQ ID NO:1) at inhibiting the proliferation of "primitive" human progenitor cells, as measured by the reduction of cells killed by exposure to high specific activity ³H-thymidine (which only affects proliferating cells).--

Please replace the paragraph beginning at page 58, line 7, with the following:

--This example illustrates the effect of CXCR4 agonists such as SDF-1 (SEQ ID NO:1) and SDF-1 polypeptide analogs on the engraftment of human cells in human fetal liver transplanted NOD/SCID mice (Figure 9). As shown in this figure, there was a lack of short-term effect of CXCR4 agonists on the frequency of different human cells present in NOD/SCID mice. In these experiments, 6 to 8 weeks post-transplanted mice were injected two times, one day apart with the test compound (SDF-1 (SEQ ID NO:1), SDF-1(1-14)-(G)₄-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021) (SEQ ID NO:23) or SDF-1(1-14)-(G)₄-SDF-1(55-67) acid (CTCE013) (SEQ ID NO:13)) and sacrificed one day

later. The frequency of the phenotypically defined human hematopoietic cells detected in the long bones (tibias and femurs) of mice was determined. Administration of 0.5mg/kg of SDF-1 had no significant effect on the number of CD45/71, CD19/20, or CD34 cells, nor on the CFC or LTC-IC. In addition, none of the human cell types were detectably affected by this schedule of CXCR4 agonist administration. This data, coupled with that of Figures 7 and 8, indicates that SDF-1 (SEQ ID NO:1), SDF-1 analogs and other CXCR4 agonists may effectively augment secondary engraftment of human progenitor cells.--

Please replace the paragraph beginning at page 58, line 26, with the following:

--This example illustrates the effect of an SDF-1 polypeptide analog CTCE0021 (SEQ ID NO:23) (10mg/kg, identified as Compound #1 in Figure 12) on the recovery of leukocytes following myeloablative chemotherapy with Ara-C (300mg/kg). In the experiment described in the example, C3Hhen mice (female) were treated with 500mg/kg Ara-C for two cycles - on days 0 and 10. During the second cycle of Ara-C dosing, Ara-C treated mice were injected with 10mg/kg CTCE0021 (SEQ ID NO:23) each day. A control was conducted with animals treated with Ara-C alone. Blood was collected from the tail vein into heparin-containing tubes at the onset of the experiment, and one day before every day following the second Ara-C dose. A total leukocyte count was determined. As shown in the graph of Figure 10, the CXCR4 agonist CTCE0021 (SEQ ID NO:23) acted to inhibit the cytotoxic effects of Ara-C and to sustain a higher level of leukocytes, illustrating the reversal of myelosuppressive effects of a chemotherapeutic regimen *in vivo*.--

Please replace the paragraph beginning at page 59, line 10, with the following:

--This example illustrates the effect of an SDF-1 polypeptide analog SDF-1(1-14)-(G)4-SDF-1(55-67)-K20/E24-cyclic amide (CTCE0021 (SEQ ID NO:23), 1mg/kg) on the recovery of leukocytes following myeloablative chemotherapy with Ara-C (500mg/kg) compared to G-CSF (Neupogen®) (Figure 11). C3Hhen mice (female) were treated with 500mg/kg Ara-C for two cycles - on days 0 and 10. During the second cycle of Ara-C dosing, Ara-C treated mice were injected with 10mg/kg CTCE0021 (SEQ ID NO:23), 10mg/kg Neupogen®, alone or together (on days -1, 0, and 1 to 3), with controls receiving no drug. Blood was collected from the tail vein into heparin-containing tubes at the onset of the experiment, and one day before and 1, 7 and 12 days following the second Ara-C dose. A total white blood cell count was obtained. The results in this example indicates that not only does treatment with CTCE0021 (SEQ ID NO:23) enhance the recovery of white blood cells following myeloablative chemotherapy with Ara-C, co-treatment with the SDF-1 polypeptide analog and G-CSF (Neupogen®) resulted in a greater recovery compared the animals treated with G-CSF alone during the early treatment phase. Furthermore, the recovery following treatment with the SDF-1 polypeptide analog was sustained compared to the G-CSF treated animals.--

Please replace the paragraph beginning at page 59, line 29, with the following:

--Figure 12 depicts the results of an experiment conducted under identical conditions, but the growth (increase in leukocyte count) relative to the number of cells counted in animals treated with Ara-C alone is illustrated. By twelve days following Ara-C administration, an approximately 7.5-fold increase in leukocytes was observed in mice treated with CTCE0021 (SEQ ID NO:23) relative to

animals treated with Ara-C alone, compared to 180% obtained in animals treated with Neupogen®.--

Please replace the paragraph (and Table 5, as previously amended) beginning at page 60, line 6, with the following:

--Table 5 shows the effect of the CXCR4 agonist, CTCE-0021 (SEQ ID NO:23), on the mobilization of leukocytes (neutrophils) in mice injected intravenously. CTCE-0021 (SEQ ID NO:23) was injected intravenously into Balb/C mice at 25 mg/kg. To obtain the data in Table 5, blood was collected through cardiac puncture and counted for the increase in white blood cells, and platelets.

Table 5. Effect of the SDF-1 agonist, CTCE-0021 (SEQ ID NO:23), on the mobilization of leukocytes (neutrophils) in mice.

Treatment day	Neutrophils (10 ⁹ /l)	Lymphocytes (10 ⁹ /l)	Platelets (10 ⁹ /l)
Day 0 (untreated)	0.968 +/- 0.311	4.78 +/- 0.88	1099 +/-50
Day 2 (CTCE-0021 (<u>SEQ ID NO:23</u>) treated)	3.159 +/- 0.761	3.15 +/- 1.075	1044 +/- 65
Day 3 (CTCE-0021 (<u>SEQ ID NO:23</u>) treated)	3.209 +/- 0.735	3.371 +/-1.113	977 +/- 152
Day 5 (CTCE-0021 (<u>SEQ ID NO:23</u>) treated)	1.592 +/- 0.961	5.325 +/- 0.771	882 +/- 88
Day 5 (untreated)	0.893 +/- 0.371	6.540 +/- 0.970	937 +/-169
Day 8 (CTCE-0021 (<u>SEQ ID NO:23</u>) treated)	2.513 +/- 2.733	4.072 +/- 1.386	1111 +/- 124

--

Please replace the paragraph beginning at page 60, line 16, with the following:

--In Table 5, CTCE-0021 peptide is represented by the following structure:
SDF-1(1-31 K20/E24-cyclic acid amide) Agonist (SEQ ID NO:23).

H2N- K- P- V- S- L- S- Y- R- C- P- C- R- F- F- G- G- G- G- L- K- W- I- Q- E- Y- L- E- K- A- L-
N- CONH2--

Please replace the paragraph beginning at page 60, line 23, with the following:

--These results demonstrate that CXCR-4 agonists, such as CTCE-0021 (SEQ ID NO:23), may be used to mobilize neutrophils (for example in patients undergoing chemotherapy to facilitate blood cell recovery). In this example, intravenous injection of the CXCR-agonist may facilitate the creation of an artificial chemotactic gradient, which may facilitate an immune response in the target tissue (in this case blood). The gradient is established when the active therapeutic compound has pharmacokinetic characteristics that facilitate an appropriate residence time in the tissue into which the compound is administered, coupled with an appropriate susceptibility to degradation *in vivo* so that the concentration of the compound decreases away from the target tissue. In alternative embodiments, the invention therefore provides methods of treating a subject comprising administering to a target tissue a labile chemokine receptor agonist or antagonist so as to create an artificial chemotactic gradient. The agonist or antagonist may for example have a plasma half life of not more than 2 hours, as is the case with CTCE-0021, or not more than 1, 3, 4, or 5 hours in alternative embodiments. One aspect of the invention provides a route of therapeutic chemokine administration which establishes an essentially uniform dosage of the chemokine receptor ligand in the target tissue, with a decreasing dosage of the chemokine radiating from the target tissue. For example, an

inhaled aerosol formulation may be used to administer a labile chemokine receptor agonist or antagonist to the lung epithelium.--

Please replace the paragraph beginning at page 61, line 21, with the following:

--Alternative embodiments of CTCE0021-like and CTCE0022-like SDF-1 analogs may include CXCR4 agonist peptides such as:
SDF-1-derived E24/K28-cyclic amide (CTCE0021-like) compounds having the formula
[RNH-Lys]XaaVSXbbSYRCPCRFF[linker]LKWIQEYLEKALN-NH₂ (SEQ ID NOS:50-52); and
SDF-1-derived K20/E24-cyclic ~~acids~~ amide (CTCE0022-like) compounds having the formula
[RCONH-Lys]XaaVSXbbSYRCPCRFF[linker]LKWIQEYLEKALN-NH₂ (SEQ ID NOS:53-55).--

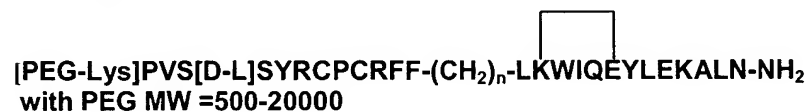
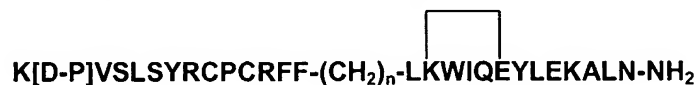
Please replace the paragraph beginning at page 61, line 30, with the following:

--In the foregoing peptides, R is a substituent that may for example be a hydrogen, alkyl, aryl or polyethyleneglycol (PEG) moiety; Xaa is an amino acid that may for example be either an L-Proline or a D-Proline moiety; Xbb is an amino acid that may for example be either a L-Leucine or a D-Leucine moiety;; and [linker] is a moiety providing a covalent attachment between the N and C terminal portions of the peptides, such as a linking moiety having 4 glycines (SEQ ID NO:211) or NH₂-(CH₂)_n-COOH (n=0-20).--

Please replace the paragraph beginning at page 62, line 6, with the following:

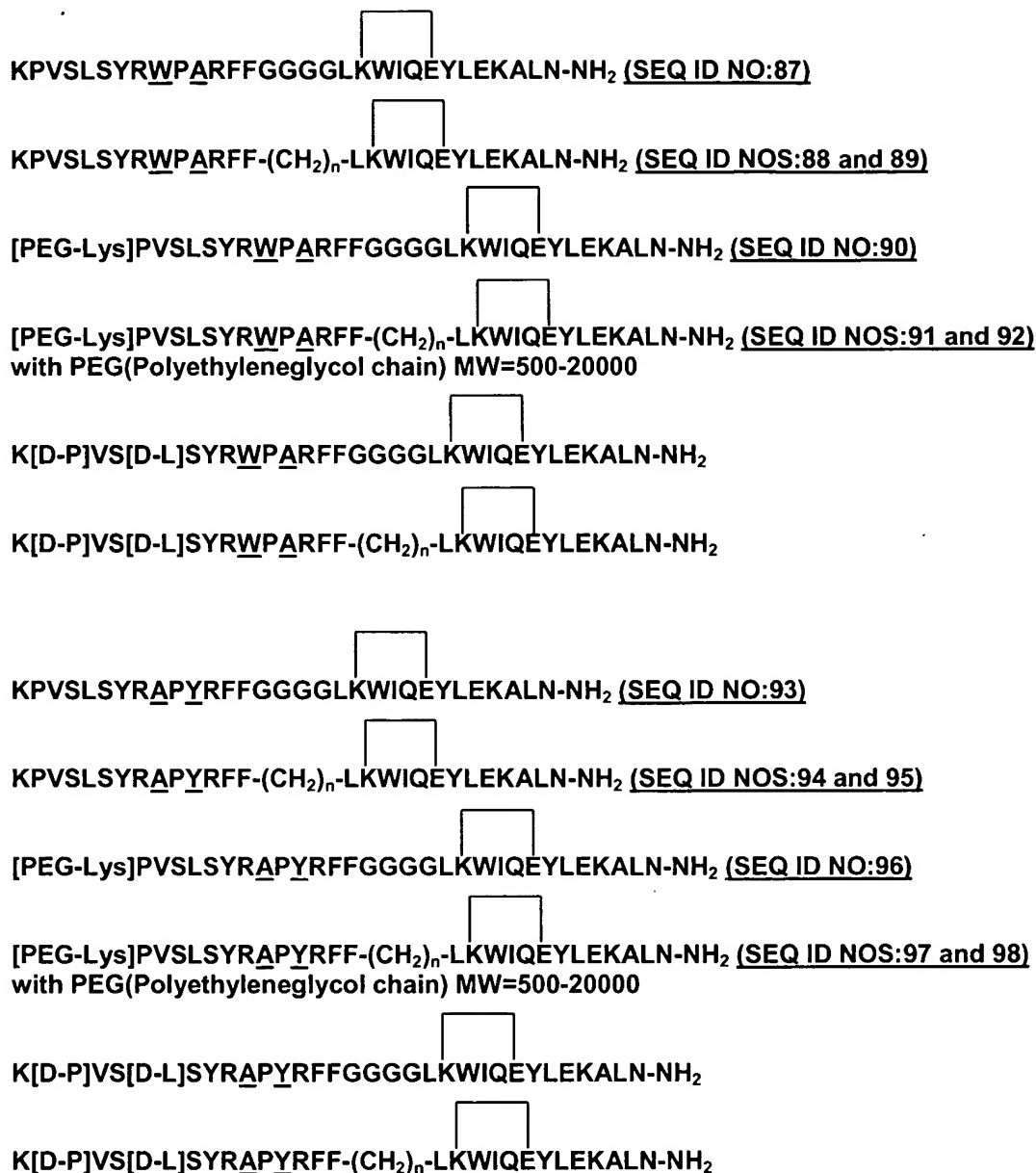
--Alternative embodiments of the forgoing peptides are as follows:

CTCE-0021-like Analogs



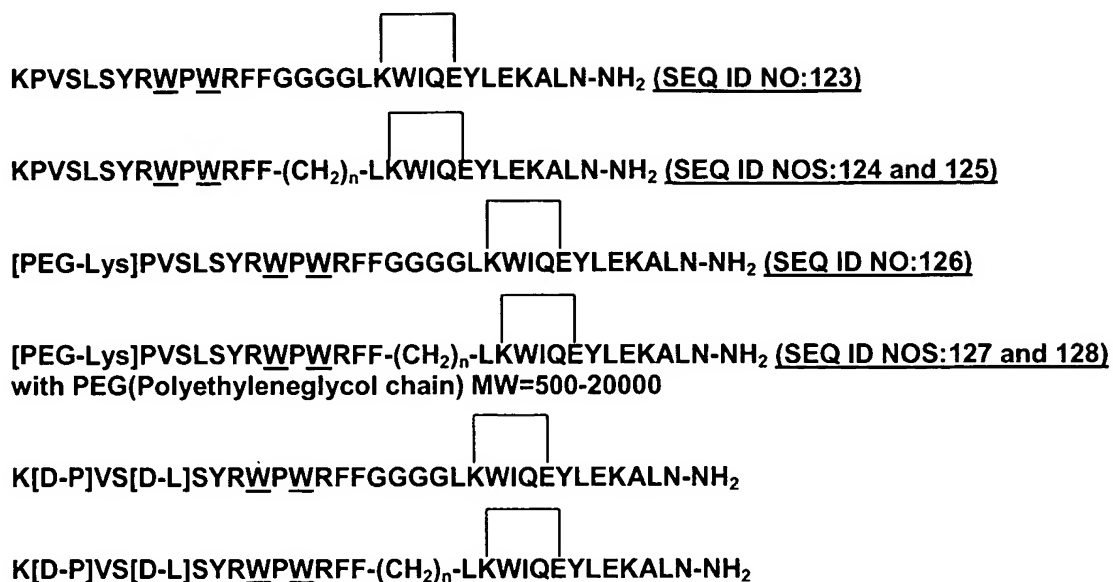




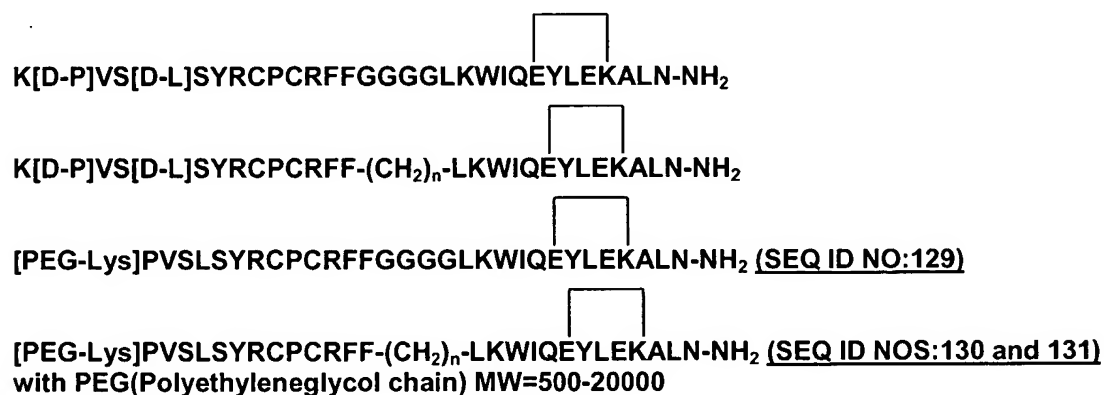








CTCE-0022-like Analogs















Structure Legend

Structure of CTCE9901:

SDF-1 (1-9)2-cysteine dimer

H2N- K- P- V- S- L- S- Y- R- C-COOH (SEQ ID NO:7)

|
S

H2N- K- P- V- S- L- S- Y- R- C-COOH (SEQ ID NO:7)

Structure of CTCE9902:

SDF-1 (1-17) mer

H2N- K- P- V- S- L- S- Y- R- C- P- C- R- F- F- E- S- H- COOH (SEQ ID NO:4)

Structure of CTCE9904:

SDF-1 (1-8)2-lysine dimer

H2N- K- P- V- S- L- S- Y- R

↘
K-CONH2 (SEQ ID NO:31)

H2N- K- P- V- S- L- S- Y- R (SEQ ID NO:32)

Structure of CTCE0013:

H2N- K- P- V- S- L- S- Y- R- C- P- C- R- F- F- G- G- G- G- L- K- W- I- Q- E- Y- L- E- K- A- L- N- COOH
(SEQ ID NO:13)

Structure of CTCE0017:

H2N- K- P- V- S- L- S- Y- R- C- P- C- R- F- F- G- G- G- G- L- K- W- I- Q- E- Y- L- E- K- A- L- N-
CONH2 (SEQ ID NO:15)

Structure of CTCE0022:

SDF-1 (1-31 E24/K28-cyclic amide) Agonist

H2N- K- P- V- S- L- S- Y- R- C- P- C- R- F- F- G- G- G- G- L- K- W- I- Q- E- Y- L- E- K- A- L- N- CONH2
(SEQ ID NO:22)

Appl. No. 10/086,177
Amdt. dated Oct. 12, 2006
Reply to Final Office Action of Oct. 12, 2005

PATENT

Structure of CTCE0021:
SDF-1 (1-31 K20/E24-cyclic acid amide) Agonist

~~H2N-K-P-V-S-L-S-Y-R-C-P-C-R-F-F-G-G-G-G-L-K-W-I-Q-E-Y-L-E-K-A-L-~~
~~N-CONH2~~

~~H2N-K-P-V-S-L-S-Y-R-C-P-C-R-F-F-G-G-G-G-L-K-W-I-Q-E-Y-L-E-K-A-L-~~
~~N-CONH2 (SEQ ID NO:23)--~~

Please cancel the present "SEQUENCE LISTING", pages 1-11, submitted September 9, 2002,
and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page
numbers 1 to 96, at the end of the application.